435/69.1
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Exo III Generated Structures

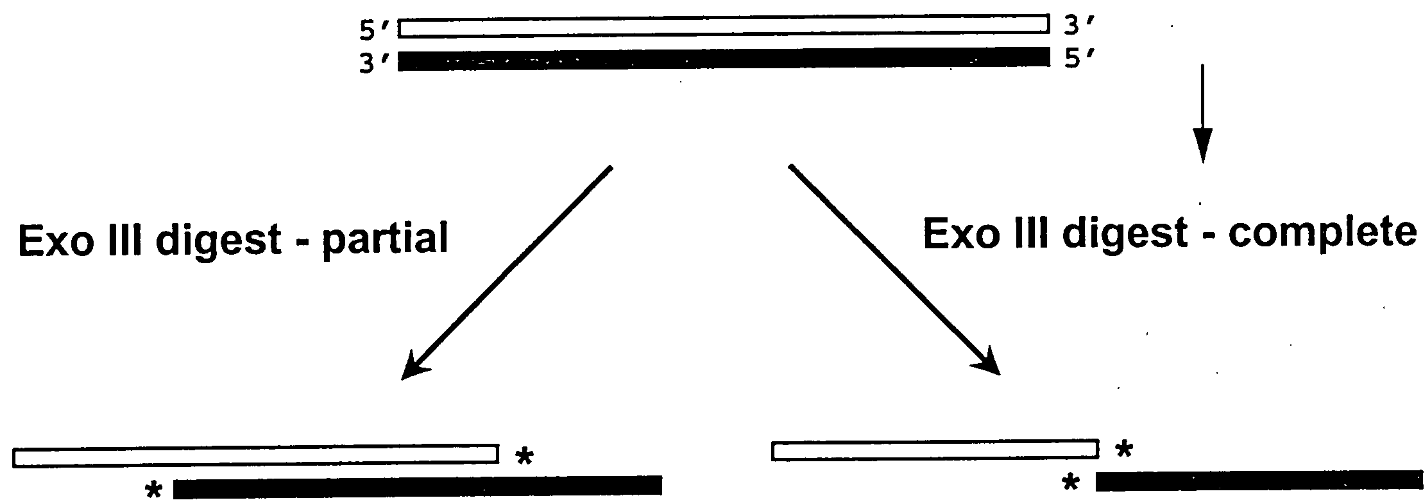


Figure 1

Figure 2. Generation of A Nucleic Acid Building Block by Polymerase-Based Amplification.

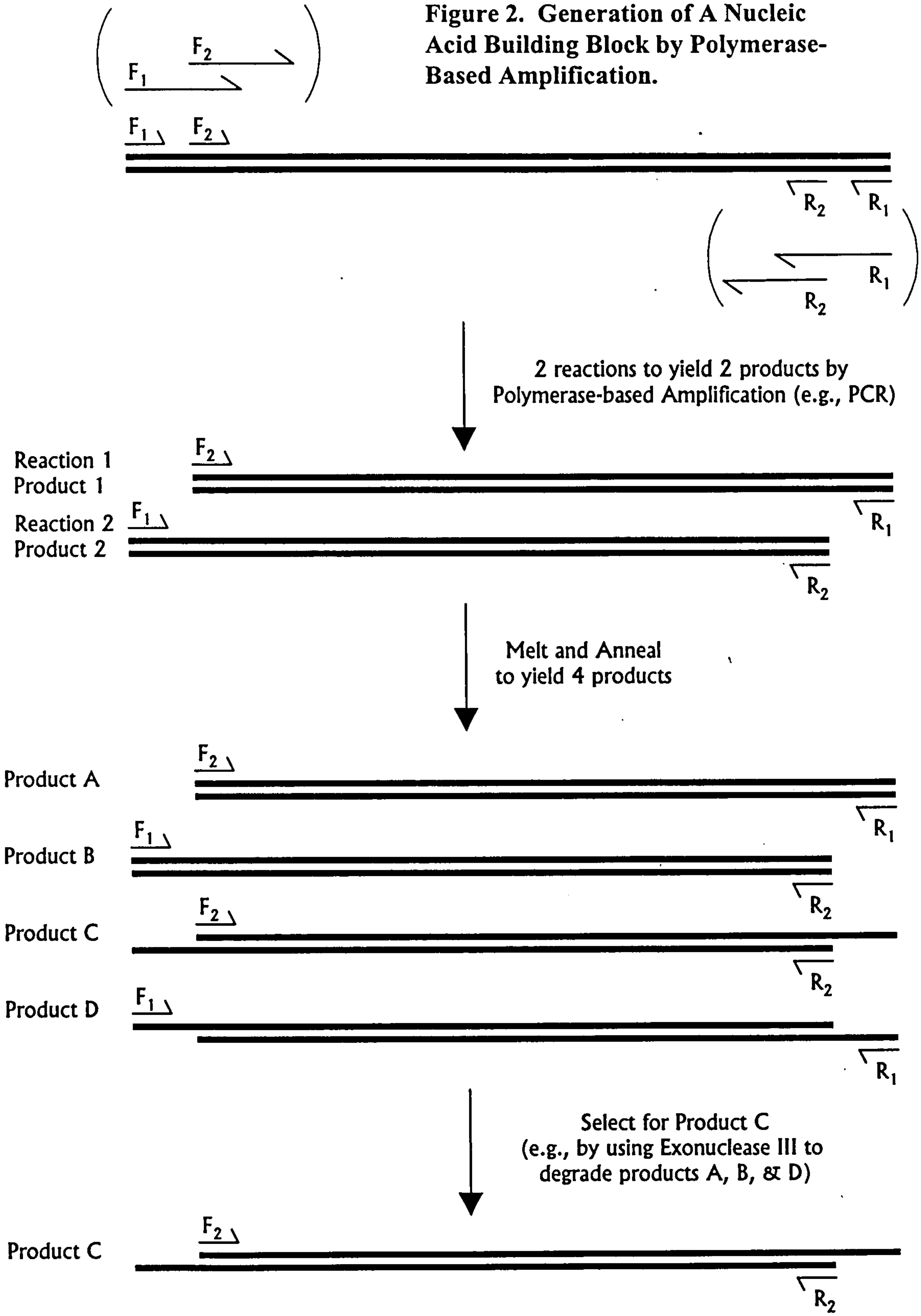


FIGURE 3. Unique Overhangs And Unique Couplings.

The number of unique overhangs of each size (e.g. the total number of unique overhangs composed of 1 or 2 or 3, etc. nucleotides) exceeds the number of unique couplings that can result from the use of all the unique overhangs of that size. For example, the total number of unique couplings that can be made using all the 8 unique single-nucleotide 3' overhangs and single-nucleotide 5' overhangs is 4.

PANEL A. 4 unique single-nucleotide 3' overhangs are possible (i.e., A, C, G, & T). For each of these there is a complementary 3' overhang with which it can pair (i.e., T, G, C, & A, respectively), as shown.

PANEL B. However, the number of unique single-nucleotide 3' overhangs is greater than the number of unique couplings. Thus, only 2 intrinsically unique couplings exist using single-nucleotide 3' overhangs as shown.

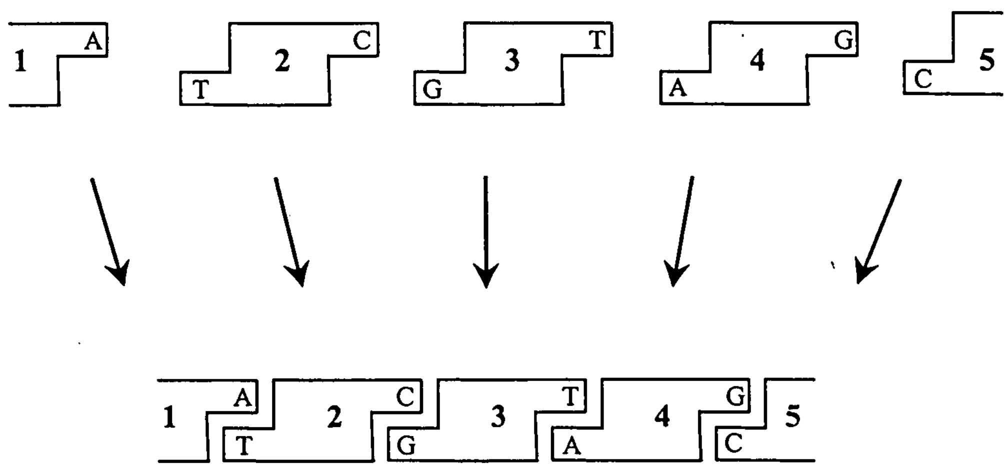
PANEL C. Likewise, 4 unique-single nucleotide 5' overhangs are possible (i.e., A, C, G, & T). For each of these there is a complementary 5' overhang with which it can pair (i.e., T, G, C, & A, respectively), as shown.

PANEL D. However, the number of unique single-nucleotide 5' overhangs is greater than the number of unique couplings. Thus, only 2 intrinsically unique couplings exist using single-nucleotide 5' overhangs as shown.

FIGURE 4. Unique Overall Assembly Order Achieved by Sequentially Coupling the Building Blocks

Awareness of the degeneracy (between the number of unique overhangs and the number of unique couplings) is important in order to avoid the production of degeneracy in the overall assembly order of the finalized nucleic acid. However, a unique overall assembly order can also be achieved - despite the use of non-unique couplings - by using building blocks having distinct combinations of couplings, and/or by stepping the assembly of the building blocks in a deliberately chosen sequence.

PANEL A. For example, one could attempt to assemble the following nucleic acid product using the 5 nucleic acid building blocks as shown.



PANEL B. However, degeneracy in the overall assembly order of the 5 nucleic acid building blocks would be present if the assembly process were carried out in one step. For example, building block #2 and building block #3 could both couple to building block #1 as shown.

Figure 5. Unique Couplings Available Using a Two-Nucleotide 3' Overhang.

16 unique 3' overhangs can be formed using two nucleotides. However, use of these 16 unique overhangs allows for the formation of only 6 unique couplings. Another 6 unique couplings are provided by the use 5' overhangs formed using two nucleotides. Thus, a total of 12 unique couplings are provided by the combined use of 3' and 5' two-nucleotide overhangs. "Twin" couplings are marked in the same shading.

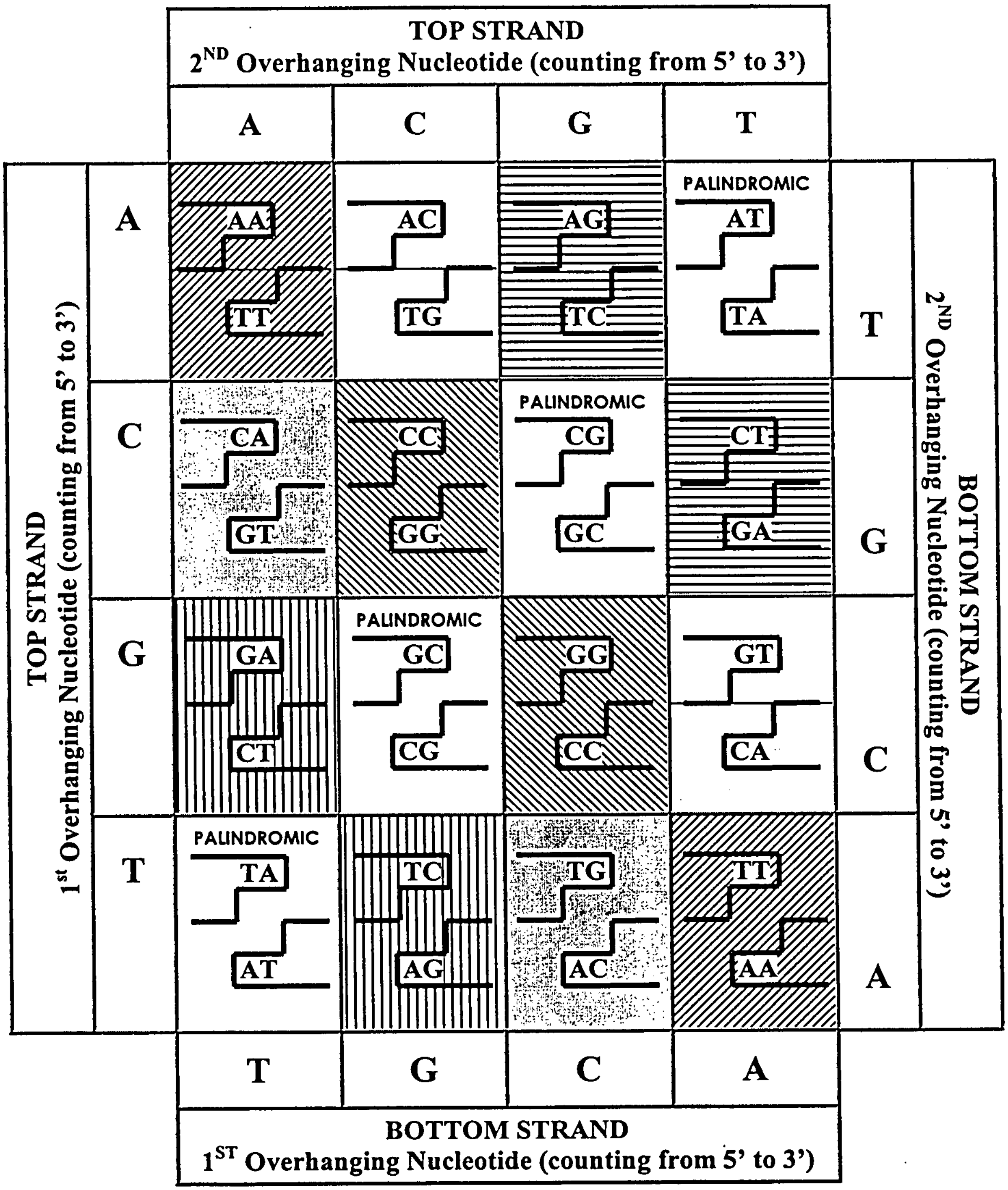


FIGURE 4 cont.

PANEL C. However, a unique overall assembly order could be achieved by sequentially coupling the building blocks in 2 steps (rather than all at once) as shown.

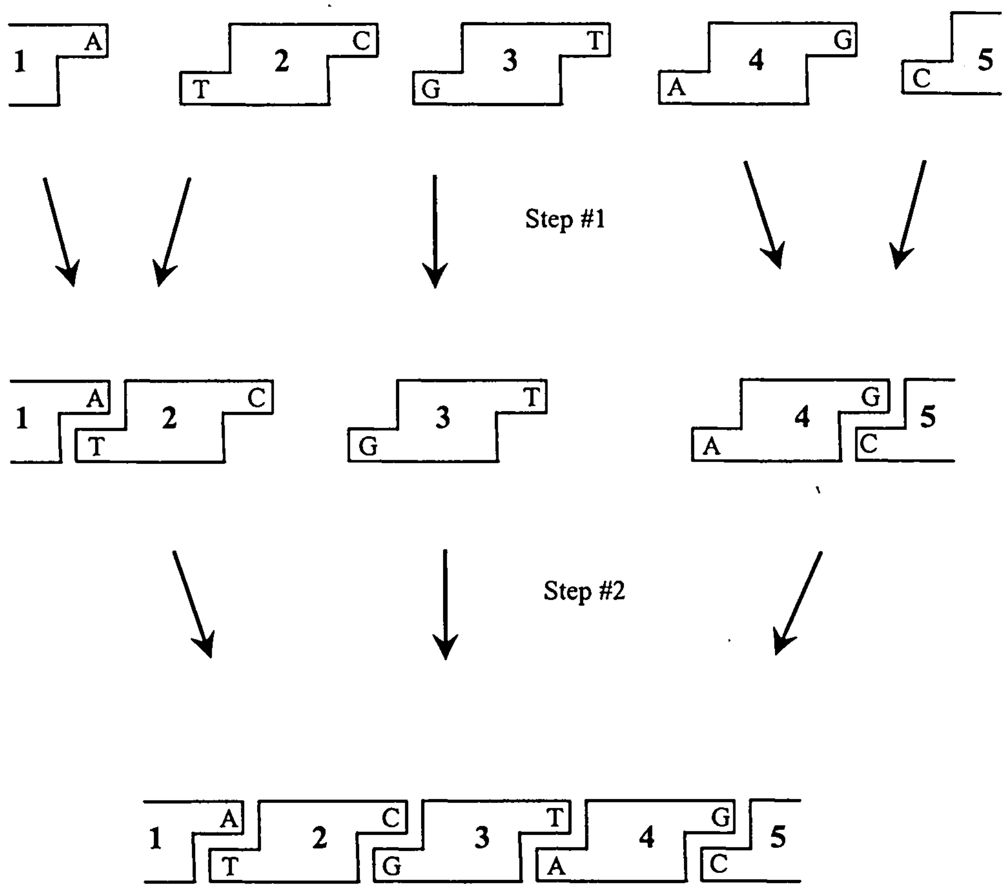


Figure 6. Generation of an Exhaustive Set of Chimeric Combinations by Synthetic Ligation Reassembly.

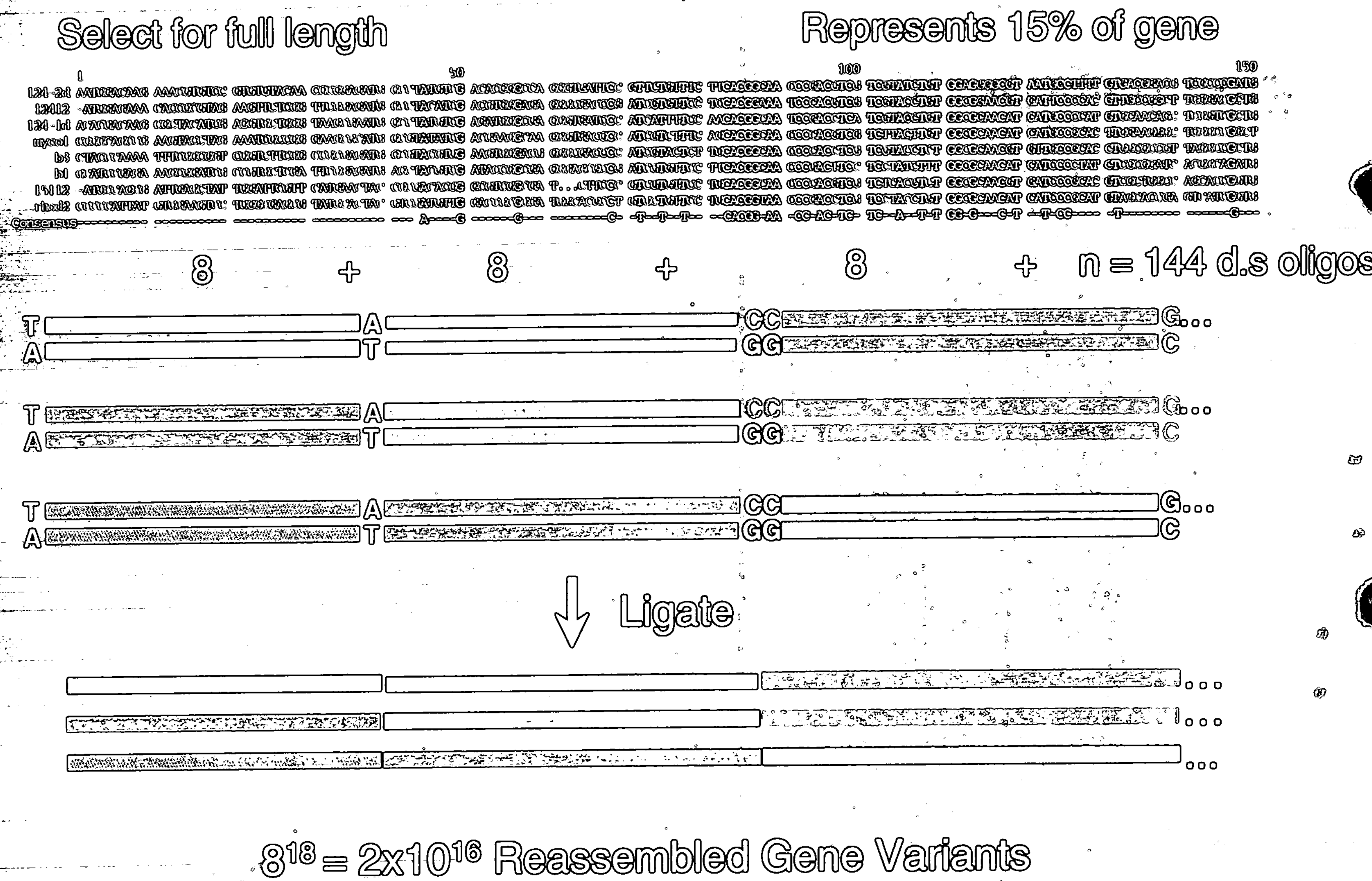


Figure 7. Synthetic genes from oligos.

```
               NcoI                                                         CCGT
150am13_00     c ATGATGCACG GCGATATTTC ATCGAGCAAT GACACGGTCG GCGTTGCCGT
150AM7_001     c ATGCATCACG GCGACATTTC ATCGAGCAAT GACACGGTCG GCGTTGCCGT
431am7_002     c ATGAGACACG GAGATATCTC CAGCAGCAAC GATTGCGTGG GCGTGGCCGT

                                                           GAG GT
150am13_00       CGTGAACTAC AAGATGCCTC GCCTTCATAC CAAGGCGGAG GTTTTAGCGA
150AM7_001       CGTGAACTAC AAGATGCCGC GGCTTCACAC CAAGGCTGAG GTGCTGGCCA
431am7_002       CGTGAACTAC AAGATGCCGC GGCTGCATAC CCGCGCGGAG GTGATGGAGA

                                                            CGG
150am13_00       ACGCCAGAAA GATCGGCGAG ATGATCGTCG GCATGAAGAC CGGCCTGCCC
150AM7_001       ACTGCCGCAA GATCGCCGAC ATGCTGGTCG GCATGAAGAG CGGCCTGCCG
431am7_002       ACGCCCGCAA GATCGCCGAC ATGGTCGTGG GCATGAAGCG CGGCCTGCCC

                                                  CCACG
150am13_00       GGAATGGATC TGGTGATCTT CCCGGAATAT TCGACCCACG GCATCATGTA
150AM7_001       GGAATGGATC TGGTGATCTT CCCGGAATAT TCCACCCACG GCATCATGTA
431am7_002       GGCATGGACC TGGTCATCTT CCCCGAGTAC TCCACCCACG GCATCATGTA

                                                     CCC GG
150am13_00       CGACTCCAAG GAAATGTACG ATACCGCGTC CGTCGTGCCC GGCGAGGAGA
150AM7_001       CGACTCCAAG GAGATGTACG ACACGGCGTC GACGGTGCCG GGTGAAGAGA
431am7_002       CGACGCCAAG GAAATGTACG AAACCGCTTC GGCCATTCCG GGCGAAGAGA

                                                       G GGG
150am13_00       CCGAGATTTT TGCCGAAGCC TGCCGCAAGG CGAAAGTCTG GGGCGTGTTC
150AM7_001       CCGAGATTTT CGCCGAGGCC TGCCGCAAGG CCAAGGTCTG GGGCGTGTTC
431am7_002       CTGCTGTGTT CGCCGACGCC TGCCGCAAGG CCAACGTATG GGGCGTGTTT

                                                     AAAG C
150am13_00       TCGCTCACCG GCGAACGTCA CGAGGAACAT CCGAAGAAGG CGCCCTACAA
150AM7_001       TCGCTGACCG GCGAGCGCCA CGAGGAGCAT CCCAATAAAG CGCCGTACAA
431am7_002       TCGCTGACGG GCGAGCGCCA CGAAGAGCAC CCGAACAAGG CGCCGTACAA

                                                      CAG AA
150am13_00       CACGCTGATC CTGATGAACG ACAAGGGCGA GGTGGTCCAG AAATACCGCA
150AM7_001       CACCCTGATC CTGATGAACG ACAAGGGTGA AGTCGTTCAG AAATATCGCA
431am7_002       CACGCTCATC CTGATGAACA ACAAGGGCGA GATCGTGCAG AAGTACCGCA

                                              GGTA
150am13_00       AGATCATGCC GTGGGTTCCG ATCGAGGGCT GGTACCCCGG CAACTGCACC
150AM7_001       AGATCATGCC GTGGGTGCCG ATCGAAGGCT GGTATCCCGG CAACTGCACG
431am7_002       AGATCATGCC CTGGGTGCCG ATCGAAGGCT GGTATCCGGG CGATTGCACC

                                        TGAAG
150am13_00       TACGTCTCCG ACGGGCCGAA GGGCATGAAG GTTTCGCTGA TCATCTGCGA
150AM7_001       TACGTCTCCG AAGGCCCGAA GGGCATGAAG ATGTCGCTGA TCATCTGCGA
431am7_002       TATGTGTCGG AAGGCCCCAA GGGACTGAAG ATCAGCCTCA TCATCTGCGA

                                  TCTGGCG
150am13_00       TGACGGCAAC TATCCGGAAA TCTGGCGCGA CTGCGCCATG AAGGGCGCCG
150AM7_001       CGACGGCAAC TACCCGGAAA TCTGGCGTGA CTGCGCGATG AAGGGCGCCG
431am7_002       CGACGGCAAT TACCCCGAGA TCTGGCGCGA TTGCGCCATG CGCGGCGCCG
```

Figure 7 cont.

```
                                      CCAG
150am13_00     AGCTGATCGT  GCGCTGCCAG  GGCTACATGT  ATCCGGCCAA  GGACCAGCAG
150AM7_001     AACTGATCAT  CCGCTGCCAG  GGCTACATGT  ATCCCGCCAA  GGATCAGCAG
431am7_002     AGCTGATCGT  GCGTTGCCAG  GGATACATGT  ACCCGGCCAA  GGACCAGCAG

                                    GC
150am13_00     GTCATCATGG  CGAAGGCGAT  GGCGTGGGCG  AATAATTGTT  ACGTCGCGGT
150AM7_001     GTGCTGATGG  CGAAAGCAAT  GGCCTGGGCC  AACAACGTTT  ATGTCGCGGT
431am7_002     GTCATGGTGT  CCAAGGCCAT  GGCGTGGATG  AACAACGTCT  ACGTGGCGGT

                              GGGCTTCG
150am13_00     TTCCAATGCC  GCGGGCTTCG  ATGGCGTCTA  TTCGTATTTC  GGCCACTCGG
150AM7_001     CGCCAATGCC  TCGGGCTTCG  ACGGCGTCTA  CTCGTATTTC  GGCCATTCGG
431am7_002     GGCCAATGCC  GCGGGCTTCG  ACGGCGTGTA  TTCCTACTTC  GGCCATTCGG

                             TTCGA
150am13_00     CGATCATCGG  CTTCGATGGC  CGCACGCTCG  GCGAATGCGG  CGAGGAAGAA
150AM7_001     CGATCATCGG  CTTCGACGGC  CGTACCCTCG  GCGAATGCGG  CGAGGAGGAT
431am7_002     CCATCATCGG  CTTCGACGGC  CGCACGCTGG  GCGAATGCGG  TGAAGAAGAC

                         C  AGTA
150am13_00     TACGGCATCC  AGTATGCCCA  GCTTTCGAAG  ATGCTGATCC  GCGACGCCCG
150AM7_001     TATGGCATCC  AGTATGCCGC  CATCTCCAAG  TCGCTGATCC  GCGACGCGCG
431am7_002     ATGGGCGTGC  AGTACGCCGA  GCTCTCCACC  AGCCTGATCC  GCGACGCGCG

                           CAATC
150am13_00     CCGCACCGGA  CAATCGGAAA  ACCATCTCTT  CAAGCTGGTG  CATCGTGGCT
150AM7_001     CCGCACCGGC  CAATCGGAAA  ACCATCTCTT  CAAGCTGGTG  CACCGTGGCT
431am7_002     CAAGAACATG  CAGTCGCAGA  ACCACTTGTT  CAAGCTGGTG  CACCGCGGCT

                           GATCAA
150am13_00     ACACCGGGTT  GATCAACTCC  GGCGAGGGCG  ACCGCGGTCT  CGCGGCCTGT
150AM7_001     ACACCGGCAT  GATCAATTCC  GGCGAGGGCG  ACCGCGGTGT  CGCGGCTTGC
431am7_002     ACACCGGCAA  GATCAATTCC  GGCGAAGAGG  CCACCGGCGT  CGCGGCATGC

                 TTA
150am13_00     CCTTATGAGT  TCTACAACAA  ATGGATCGCC  GATCCGGAAG  GCACCCGCGA
150AM7_001     CCGTATGATT  TCTATTCGAA  ATGGATCGCC  GATCCCGAGG  GTACACGCGA
431am7_002     CCGTACAACT  TCTACGCCAA  CTGGATCAAC  GATCCGGAGG  GCACGCGCAA

                ATGGT
150am13_00     AATGGTCGAG  TCCTTTACCC  GGCCGACGGT  GGGAACCGAT  GAAGCGCCCA
150AM7_001     GATGGTGGAA  TCCTTCACGC  GTCCGACGGT  GGGTGTGGAG  GAATGCCCGA
431am7_002     GATGGTCGAA  TCCTTCACCC  GGTCCACCGT  GGGCACGCCG  GAGTGCCCCA

               TCGAG
150am13_00     TCGAAGGCAT  CCCGAACAAG  GTCGCGGTGC  ACCGCTGA     aagct
150AM7_001     TCGAGGGCAT  TCCGAACAAG  GCCACCACGC  ACCGCTGA     aagct
431am7_002     TGGACGGCAT  CCCCAACGAG  GACGCCAAGC  ACCGCTAG     aagct
                                                                HindIII
```

Figure 8. Nucleic acid building blocks for synthetic ligation gene reassembly.

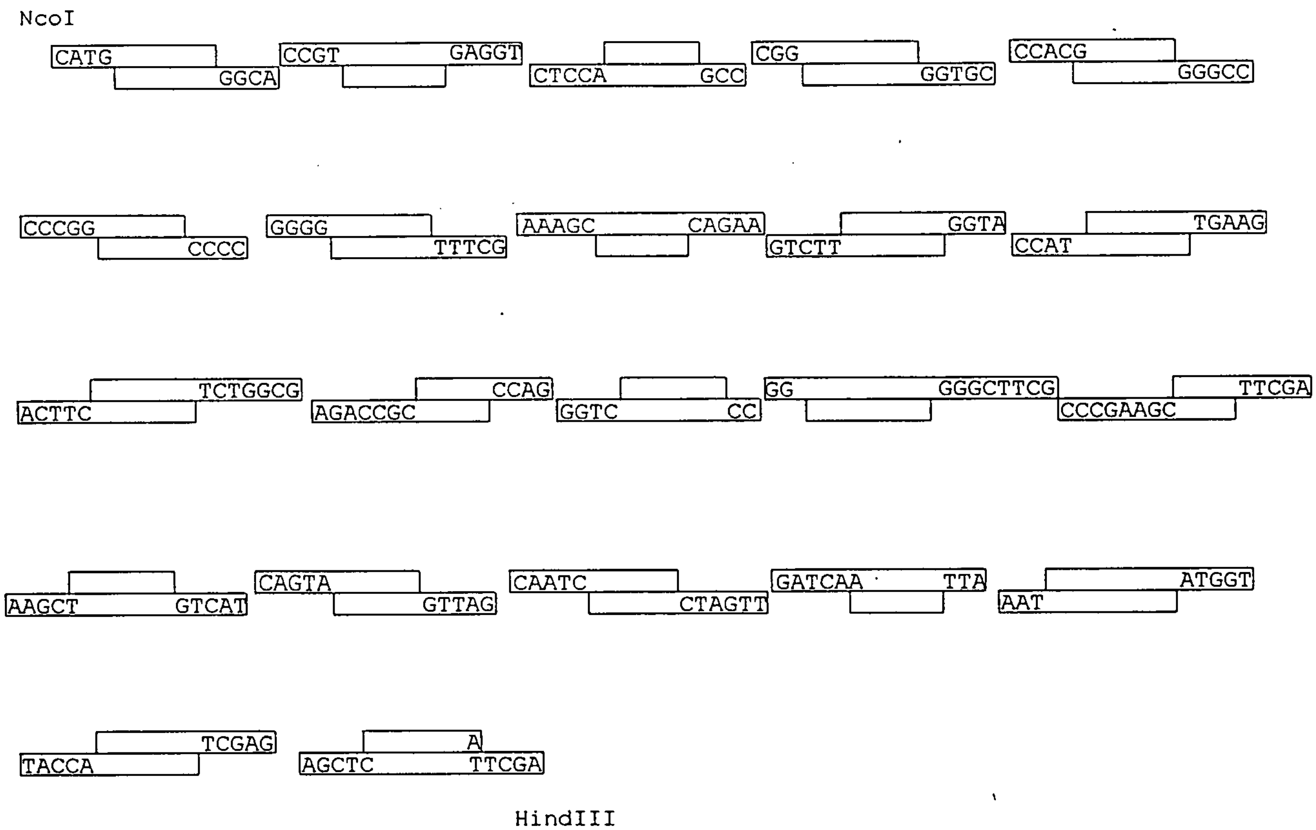
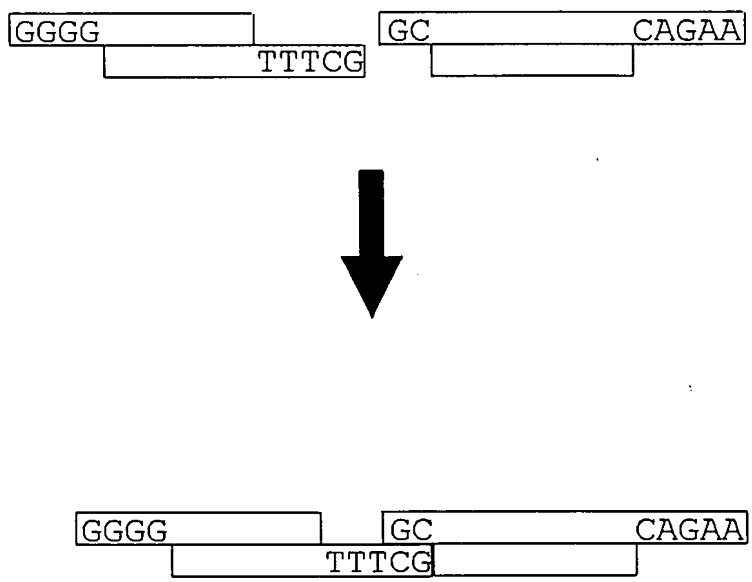


Figure 9. Addition of Introns by Synthetic Ligation Reassembly.

Figure 10. Ligation Reassembly Using Fewer Than All The Nucleotides Of An Overhang.

Gap Ligation



Ligation of one strand only;
gap in second strand can be repaired in vivo

Figure 11. Avoidance of unwanted self-ligation in palindromic couplings.

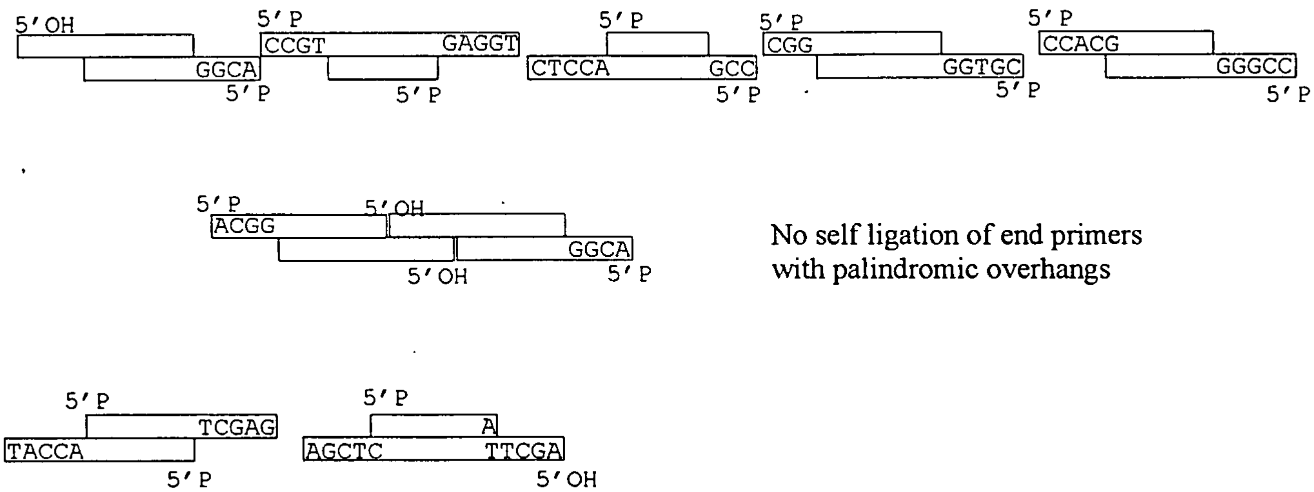


Figure 12. Pathway Engineering

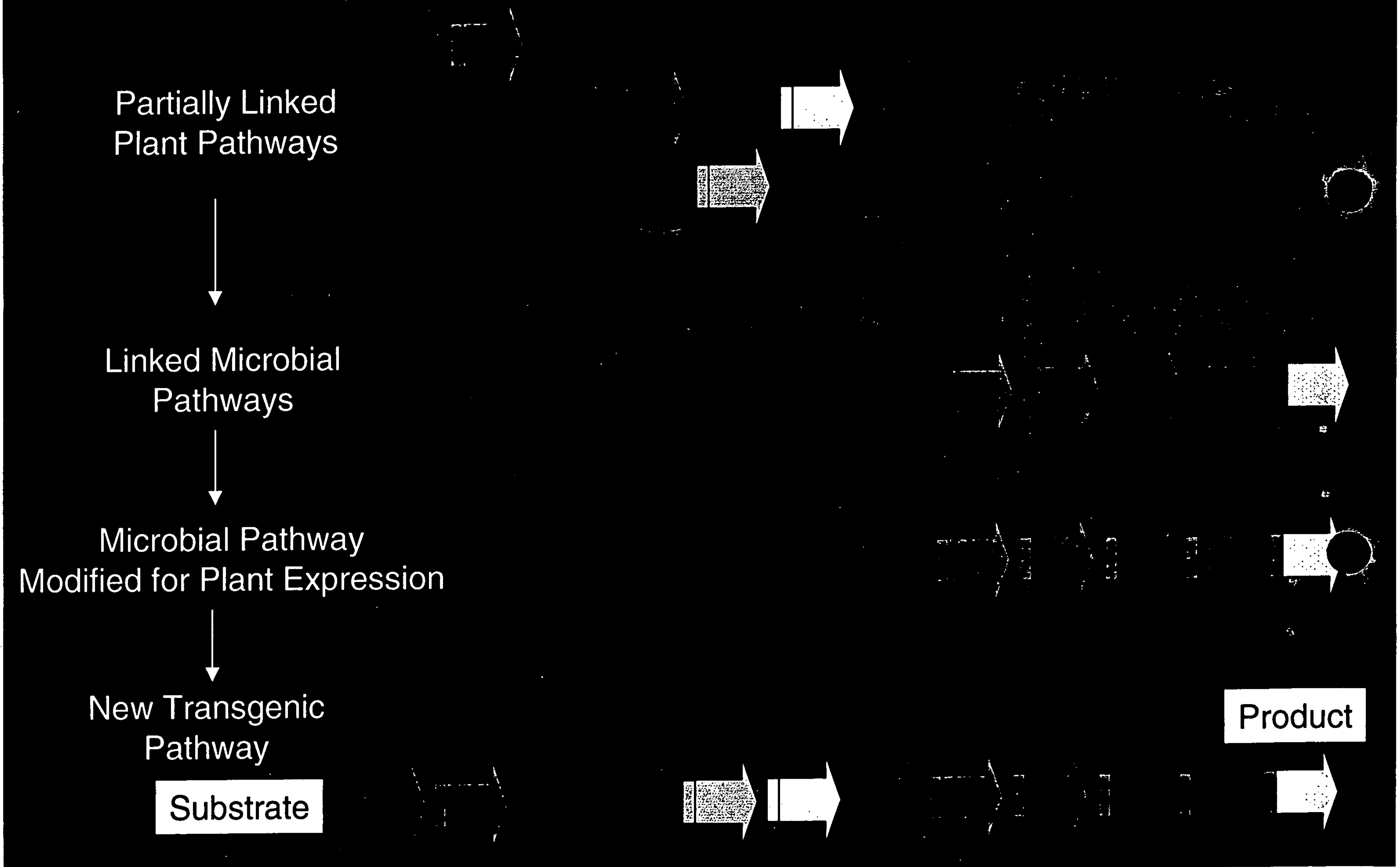


Figure 13. Pathway Improvement / Evolution

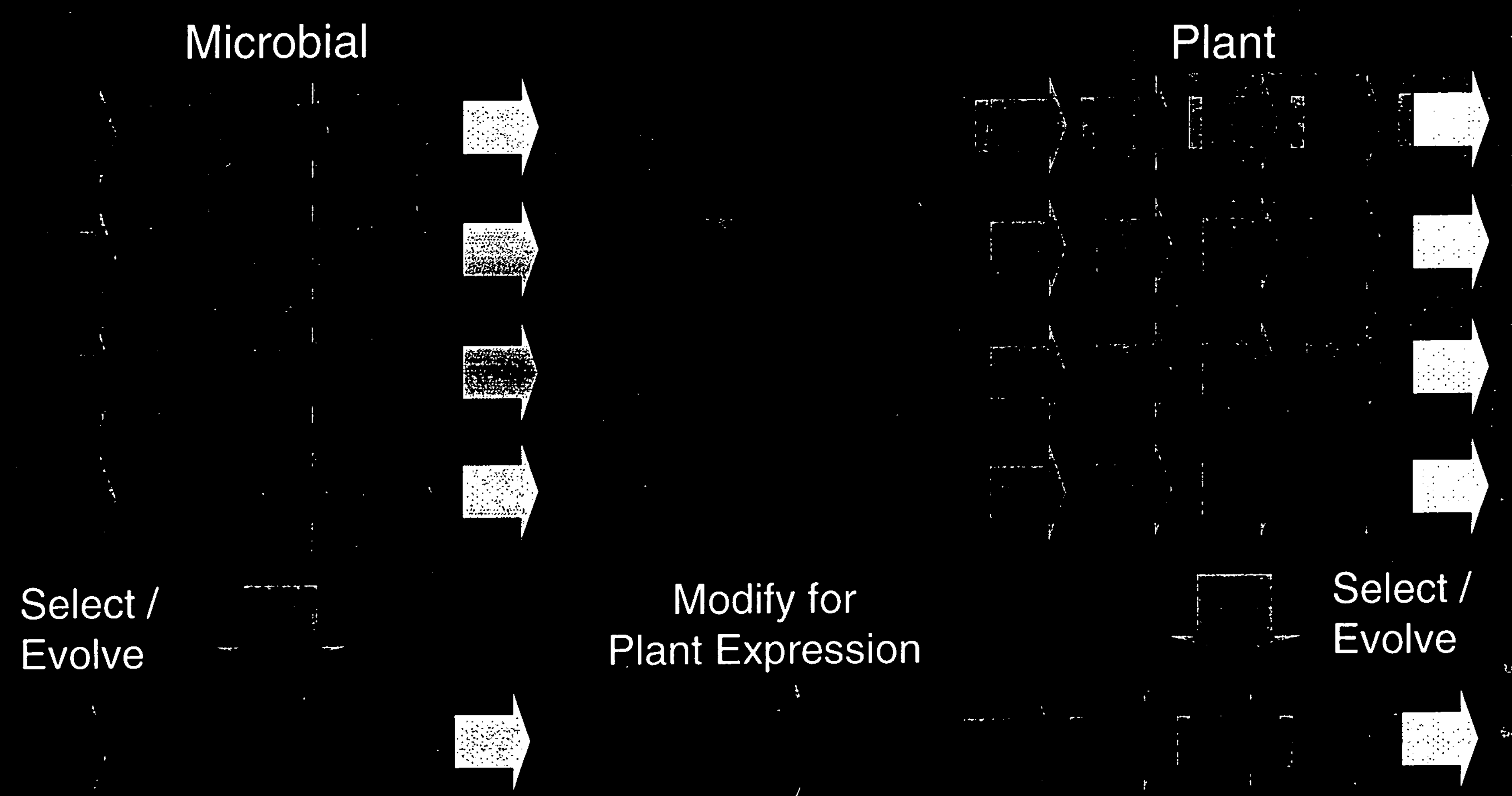


Fig 14. Conversion of Microbial Pathways to Eukaryotic Pathways

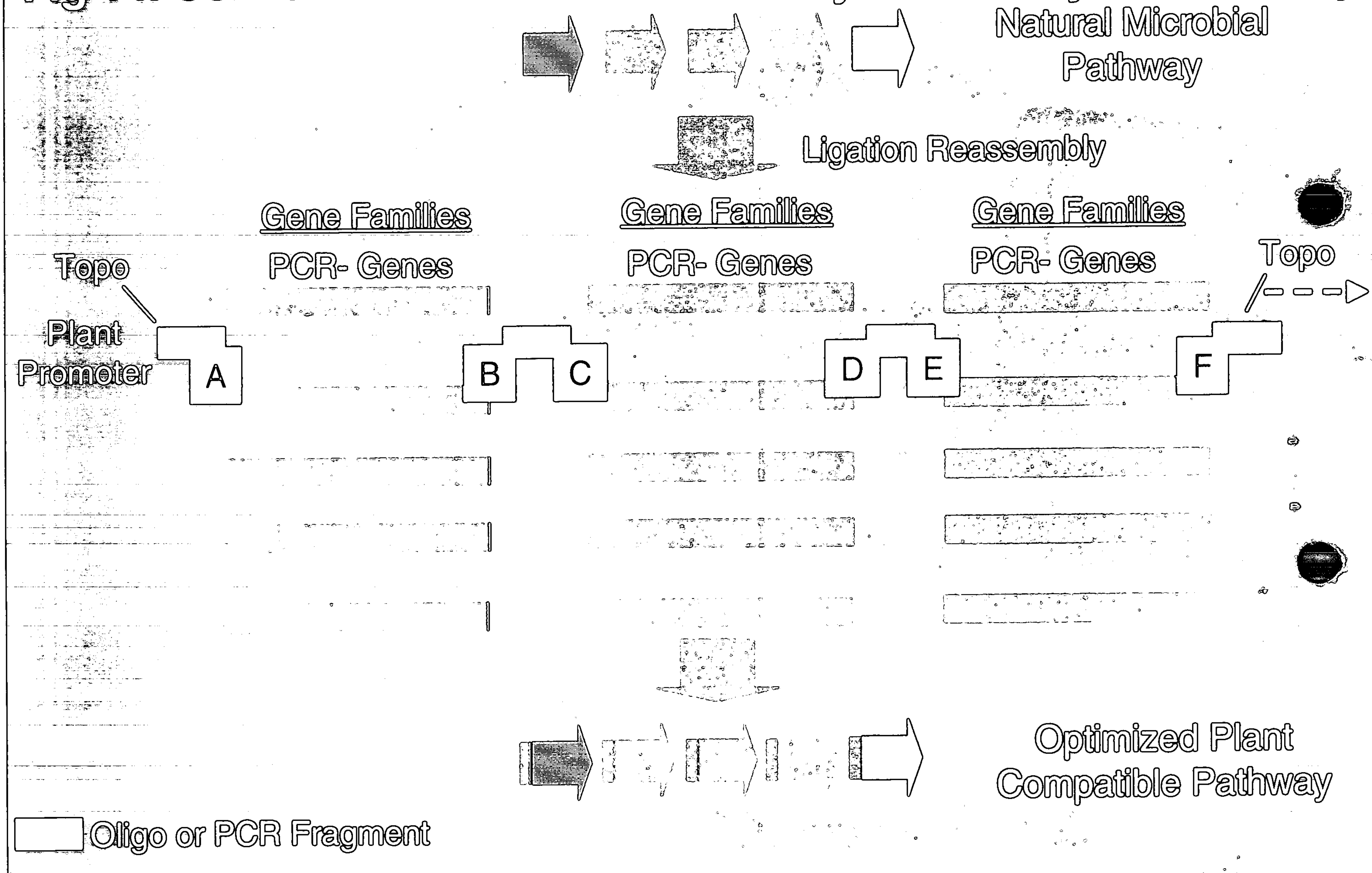


Figure 15

Evolution of polypeptides by synthesizing (in vivo or in vitro) corresponding deduced polynucleotides and subjecting the deduced polynucleotides to directed evolution and expression screening subsequently expressed polypeptides.

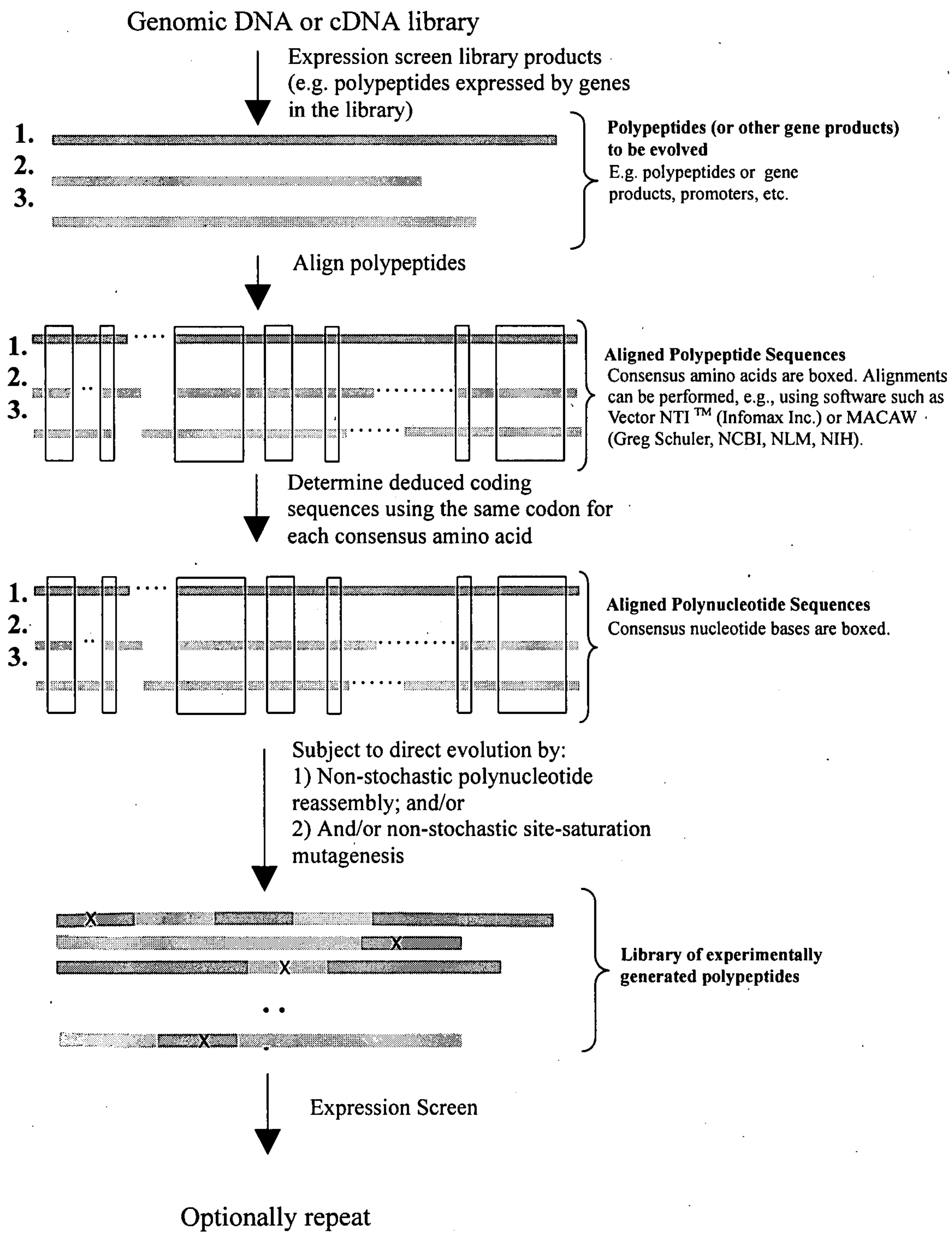
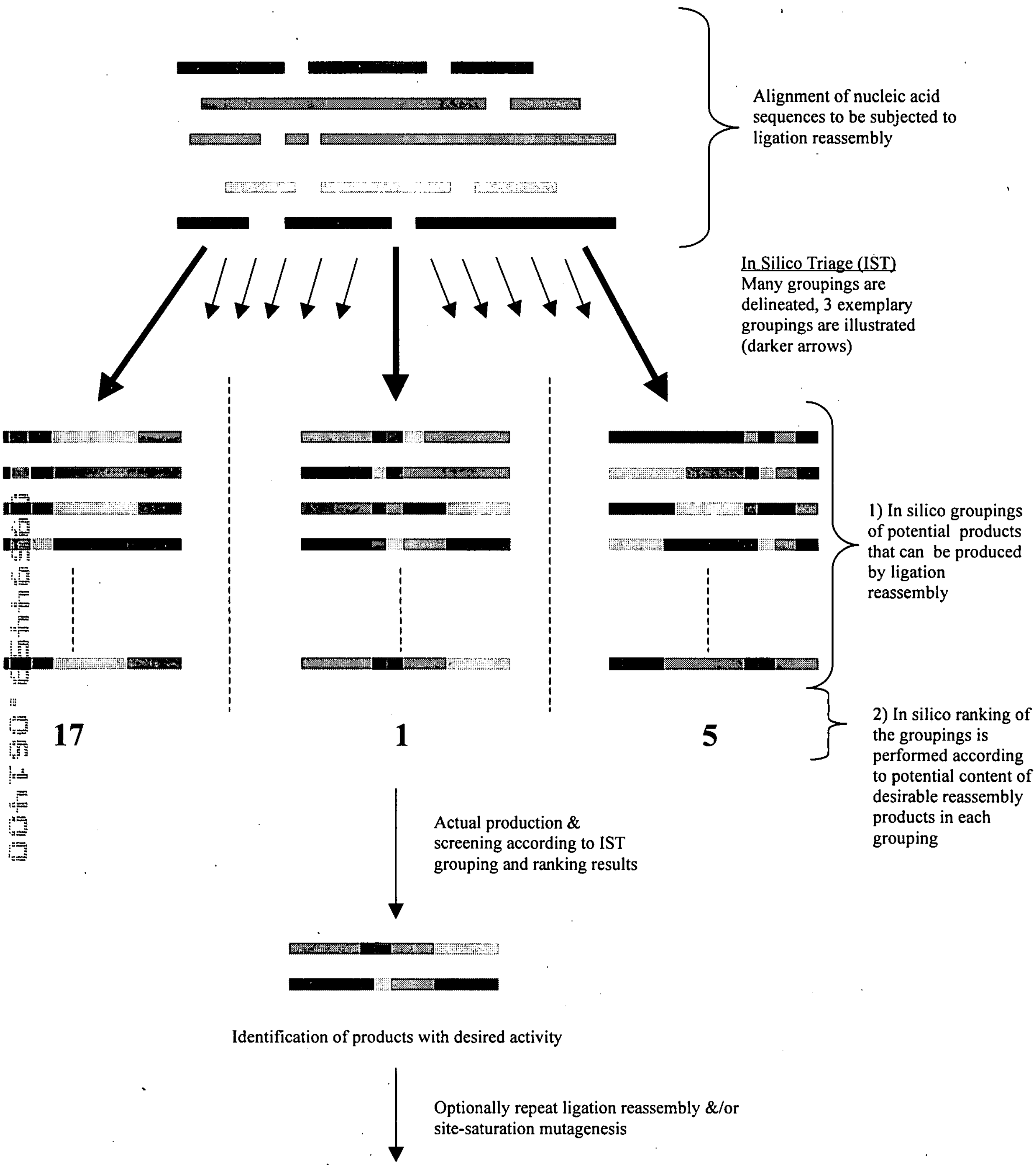
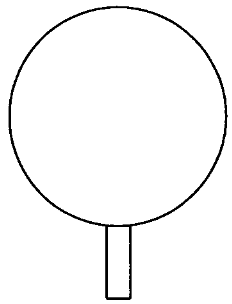


Fig. 16: In Silico Triage





Streptavidin-conjugated
Magnetic beads

```
                                          F1                     F2                        F3                    F4
                                          85-bp                  49-bp                     36-bp                 48-bp
5'Biotinyl-spacer-TOPO sequence-AAGGTATTG--------GGCCTTC CGGACCGC----------TTCATCGAG CAGGTGCT-------CCGCTTACG GAGGTCGAG--------
                                     ATAAC--------CCGGAAGGCC TGGCG----------AAGTAGC TCGTCCACGA-------GGCGAATG CCTCCAGATC--------
GGACAACT

                              F5
                              45-bp
   ---CCTGTTGACCGA GAGCCACTG--------GCCGGTGAG
                  GGCTCTCGGTGAC--------CGGCCACTCGGGC
```

Outline of the procedure

1. Annealing of complementary oligos.
   (Couplings are shown underlined & in bold. Dashes "------" indicate internal sequences not involved in couplings.
2. Immobililization of 5' pre-annealed biotinytated fragment to conjugated beads.
3. Wash to remove free fragments.
4. Enzymatic ligation of consecutive pre-annealed fragments including washes between each addition to remove free fragments.
5. BsaI-mediated elution of reassembled gene (cuts inside the TOPO sequence).
6. Ligation to 5'- and 3'end PCR generated fragments (if necessary).
7. Cloning into appropriate vector.

Fig. 17: Solid Phase Ligation Reassembly

Figure 18

Non-stochastic polynucleotide reassembly in combination with non-stochastic polynucleotide site-saturation mutagenesis.

Shown below is a non-limiting example of a permutation of the directed evolution methods described herein

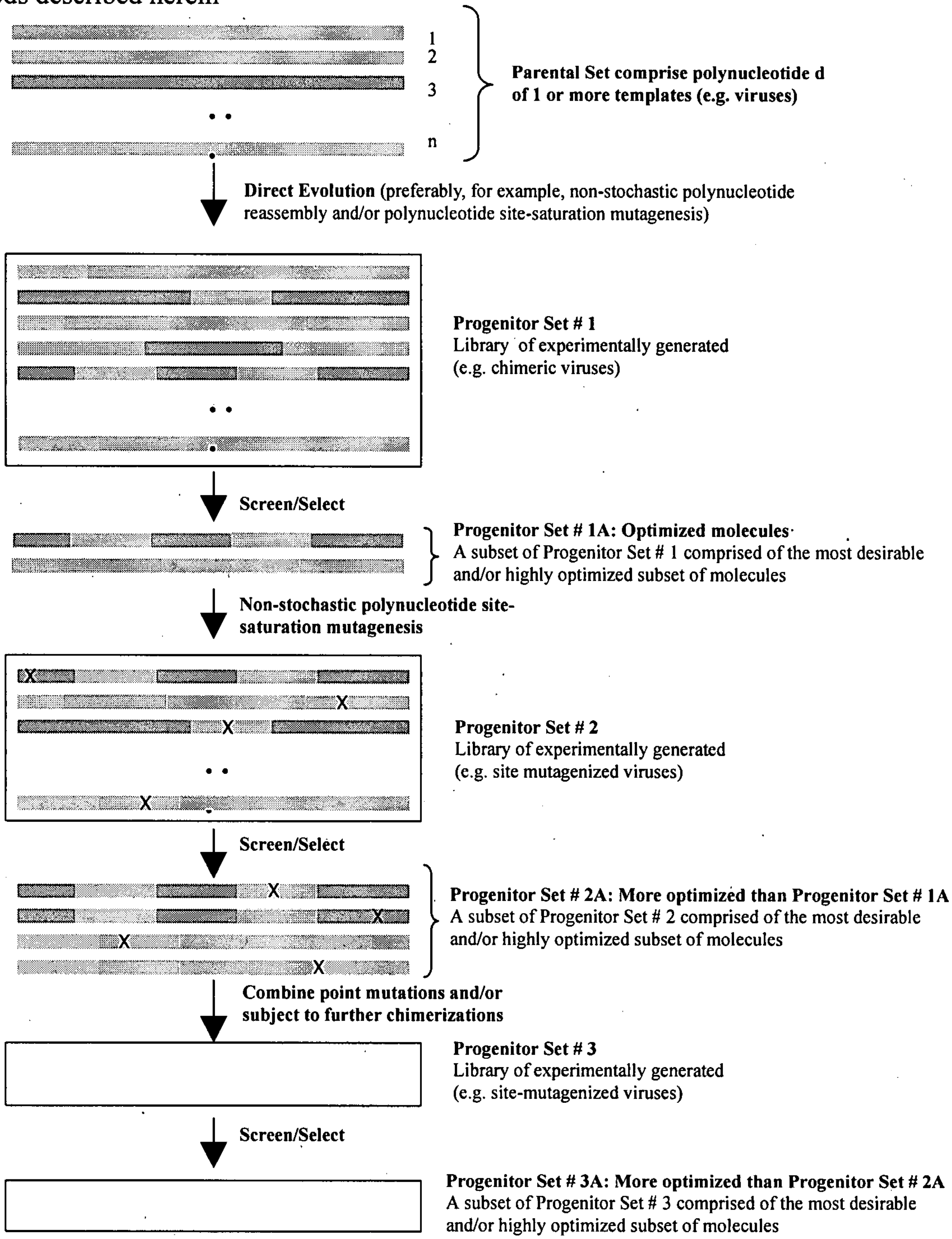


Figure 19A An alignment of two CMV-derived nucleotide sequences from human and primate species.

```
                         1                                                50
AF078102 Rhesus     (1)  ATCGATTTAAACTGCCCGATTGAGGTTGTGGCTGAACAGTTTGTGAGTC-
 M67443 Towne       (1)  --------------------------GCCATGGCATCCGTACTGGGTCC

                         51                                              100
AF078102 Rhesus    (50)  -TTTTTCGAAGGTAGATATGACACTGCTTTCCAGAAGTGCCGCTGAAGCCT
 M67443 Towne      (24)  CATTTCGGGGCACGTGCTGAAAGCCGTGTTTAGTCGCGGCGAC-ACGCCG

                         101                                             150
AF078102 Rhesus    (99)  GGGGTACATATCTGAAGCAGCGAGATGAGCGTCCCAGGACGTG-ATAGG
 M67443 Towne      (73)  GTG--------CTGCCGCA-CGAGACGCGACTCCTGCAGACGGGTATCCA

                         151                                             200
AF078102 Rhesus   (148)  G-TGCCACTACGGG-GTGTTCAGTTTGGGTAATCTCCTGTTCCAACGTTG
 M67443 Towne     (114)  CGTGCCCGTGAGCGAGCGCTCGCTGATGCTGGTGTCCCAGTACACGCCCC

                         201                                             250
AF078102 Rhesus   (196)  TCT-CAC--TATGTTACAG-GGTGTTTATGTGGGACAATACGATGTAACCG
 M67443 Towne     (164)  ACTCGACGCCATGCCAGCGCGGCGACAATGAGGTGCAGGTGCAGCACACG

                         251                                             300
AF078102 Rhesus   (242)  TG-TTTACGAGAATATATTAAAAAGAATTCTGGTAGTATCATTCCGTTGC
 M67443 Towne     (214)  TACTTTACGGGCAGCGAGGTGGAGAACGTGTCGGTGAA------CGT-GC

                         301                                             350
AF078102 Rhesus   (291)  GCAACGCGTGTAGGTGGTTTCTCGTCCTTCGGTCTGGTATAGATAATCCC
 M67443 Towne      (257)  ACAACGCCACGGCCGGA------------GC----------ATCTGCCC

                         351                                             400
AF078102 Rhesus   (341)  CAGGTAGAAACCCGT-TCGTTCGACCAGTAT-GTTTGCTGCAAAGACAAA
 M67443 Towne      (285)  CAGCCAAGAGCCCATGTCGATCTATGTGTACGCGCTGGCGGTCA-AGATG

                         401                                             450
AF078102 Rhesus   (389)  TTGGAAGCAGTGGGAATATTGGGGTGCAGTTGGGTGATGGGAGT-GATG
 M67443 Towne      (334)  CTGAACATCGCCAGCATCAACGTGCACCACTACCC-GTCGGCGGCCGAGC

                         451                                             500
AF078102 Rhesus   (438)  GCAAAAAAATCCAGGAGAGTACGTGTGTGGTTCTGCTGGGAAGGTATGGA
 M67443 Towne      (383)  GCAAACACCGACACCTGCGCGTAGCTGACGCTGTGATTC-ACGGTCGGG

                         501                                             550
AF078102 Rhesus   (488)  TTTGTGTACGTGTATGAGTGG---GACAGTGACGG-ACTCTTTCAGATAG
 M67443 Towne      (432)  CAAGCAGATGTGGCAGGCGCGTCTCACGGTCTCGGGACTGGCCTGGACGC

                         551                                             600
AF078102 Rhesus   (534)  GT-ACGTCACTGAAAGAACTCGCGGACCACGGTTTACTG-ACATGTGAAT
 M67443 Towne      (482)  GTCAGGAGAACCAGTGGAAA-GAGGCCGACG-TCTACTACAGGTCAGCGT

                         601                                             650
AF078102 Rhesus   (582)  CGGTATATGGCCATGCGGAAACGCGCTTCTCGACCAGCGAGGCTCGTTTT
 M67443 Towne      (530)  TCGTGTTTG-----CCAGCAAGGACGTGG--CAGTGCGGCAGGTGGTGTG

                         651                                             700
AF078102 Rhesus   (632)  CAAGTGGAGAAATTGTTGTGTCTCGATCCGACAGACGCACGTGGTGTAGC
 M67443 Towne      (573)  CGCG-CACGAGCTGGTT-TG-CTCCATGGAGAACACGCGCGCAACGAAGA
```

Figure 19B

```
                               701                                              750
AF078102 Rhesus      (682)     TAAGACCGCAGAAGAGTTTCATGGCGTTAACGTAGTAGTGAAAACGC-GT
   M67443 Towne      (620)     T--GCAGGTGATAGGTGACCAGTACGTCAAGGTGTACCTGGAGTCCTTCT

                               751                                              800
AF078102 Rhesus      (731)     GGCCGATCGGAGGCCGATCCCTTGCTGCTTCTGG--GTACTGTGGAGAAG
   M67443 Towne      (668)     GCGAGGACGT-GCCGTCCGGCAAGCTGTTTATGCACGTCACGCTGGGCTC

                               801                                              850
AF078102 Rhesus      (779)     CTACGTCAAATGTATCGCTTTGCTAAAATGAGAGGTGATAAGTTTAGTAA
   M67443 Towne      (717)     TGACGTGGAAGAGGACGTGACGATGACCCGCAACGCGCAACGCTTCATGC

                               851                                              900
AF078102 Rhesus      (829)     TGTG--ATAAAGTATATTAATCAACGCATGTGTTGCGGA------TGGTA
   M67443 Towne      (767)     GGCGCCACGAGCGCAACGGCTTTACGGTGTTGTGTCGCAAAAATATGATA

                               901                                              950
AF078102 Rhesus      (871)     --CGTAGTTGGTGTTA-CTGGG-CGTTACGCTAATTTTGGAATTTTGCTG
   M67443 Towne      (817)     ATCAAAGCGGGCAAGATCTCGGACAGATCATGCTGGATGTGGCTTTTACCTC

                               951                                             1000
AF078102 Rhesus      (917)     AGCTGTGGAATAATTCTGTTGGACGAGTGGGCGTGTGTTATGTGG-TGA
   M67443 Towne      (867)     AGACGAGCATT--TTGGGCTGCT-GTGTCGCAAGAGCATCCCGGGCCTGA

                               1001                                            1050
AF078102 Rhesus      (966)     GAATCGACGAAAGGGACGTGTTT--CGTCTAGGCGATAAGATGGAAATGT
   M67443 Towne      (914)     GCATGTCAGGTAAGCTATTGATGAACGGGCAGGACAT--CTTCCTGGAGG

                               1051                                            1100
AF078102 Rhesus     (1014)     TGTTCAGATGCGGCTTTGTTAAGCTGCGTGGCATTACGCGATTTGACCGC
   M67443 Towne      (962)     TGC----AAGCGA--TACGCGAGA-CCGTGGAACTGGGTCAGT--AC---

                               1101                                            1150
AF078102 Rhesus     (1064)     GGATTGCGTGGCGAGGCTGGTCTG----GAGAGTGAGT-GCTACTGGTAT
   M67443 Towne     (1000)     -GATCCCGTGGCTGCGCTCTTCTTTTTCGATATCGACTTGCTGCTGG-AG

                               1151                                            1200
AF078102 Rhesus     (1109)     GACGGTGCCCGTAAGCAAGATGTGTGTTGGTGGTGTATGGTCAACACAGT
   M67443 Towne     (1048)     CGCGGGCGTCAGTACAGCGAAGAGCCCACCTTCAGCA--GCCAGTATCGC

                               1201                                            1250
AF078102 Rhesus     (1159)     TACGGCCGAGGAGCTCTGTGGTGCGT-AGGAGTGGCTCAGGCGGAGTAAT
   M67443 Towne     (1096)     ATGGA--GGGCAAGCTTGAGTACCGACAGACCTGGG--AGCGGCACGACG

                               1251                                            1300
AF078102 Rhesus     (1208)     CGCGCTGATGATAAAATACGTAGCAGTTGTTGTTGGGGACGTAGTGATTT
   M67443 Towne     (1142)     AGGGTGGCGCCCAGGGCGAGGACGACGTCTGGACCAGCGGATCGGACTCC

                               1301                                            1350
AF078102 Rhesus     (1258)     GATGCCTACCGGAGTATTGCAAGAGAATCAGAATTGGTGGTTTCGGTGGA
   M67443 Towne     (1192)     GACGAGGAAGTC-GTAACCACCGAGCGCAAGACGCCCCGGGTTAGCGGCG

                               1351                                            1400
AF078102 Rhesus     (1308)     GGAGTGTCACGGTTCTAGAAGGGCG-GCAAAGCGGTGCGTGGCAA--GAA
   M67443 Towne     (1241)     GGGGCGCCATGG-----CGGGCGCCTGCACTTGCGCGGGCCGCAAACGCA
```

Figure 19C

```
                                1401                                               1450
AF078102 Rhesus       (1355)    AATGATGAGTTAACGGAGAAAACGTCTGATGGTACTGTGATGTTTCCTGT
   M67443 Towne       (1286)    AATCAGCA-TCCTCGGCCGACGGCGTGCACGCCGGGCGTTATGACACGCCG

                                1451                                               1500
AF078102 Rhesus       (1405)    A-GACAGAGGTTTTTCTGCCAGAACCGTAAAGTTCGAGGGTGAAAGCGAT
   M67443 Towne       (1335)    CCGCCGTTAAGGCCGAGTCC----ACCGTCGCGCCCGAAGAGGACACCGAC

                                1501                                               1550
AF078102 Rhesus       (1454)    GACTACACAGAAGAGGAGGAAGATGATGATAACGAGTCAGAAGAAACCCC
   M67443 Towne       (1381)    GAGGATTGCGACAACGA--AATCC----ACAATGCGGCCGTGTTCACCTG

                                1551                                               1600
AF078102 Rhesus       (1504)    TCAGCATCAGCCTGTTGTCATTGTTTCAGATGAAACAAACGGCAGTGTTG
   M67443 Towne       (1425)    GCCGCCCTGGCAGGCCGGCATCCTGGCCGCAACCTGGTGCCCATGGTTG

                                1601                                               1650
AF078102 Rhesus       (1554)    ATTCTTATGATGATGACAGTGACTCAT-CTCTG--TCCT-CAGATACCCA
   M67443 Towne       (1475)    CTACGGTTCAGGGTCAGAATCTGAAGTACCAGGAGTTCTTCTCGGACGCC

                                1651                                               1700
AF078102 Rhesus       (1600)    TGATGCACCACCCCCAGACATTATGGCCATAATCCAAAAGCAATGGCA--
   M67443 Towne       (1525)    --AACGACATGTACGG--CATCTTCGCCGAATTGGAAGGCGTATGGCAGC

                                1701                                               1750
AF078102 Rhesus       (1648)    ---------AAACGAAGAGCCCGTTACCAATCAAAGAGAATTGTTACTGC
   M67443 Towne       (1571)    CCGCTGCGCAACCCAAACGTCGCCGCCACCGGCAAGACGCCT-TGCCCGG

                                1751                                               1800
AF078102 Rhesus       (1689)    GGCGTGCA---CGACGA-GCTAAACATATGAAAAGTGGCATTGTTCCCT
   M67443 Towne       (1620)    GCCATGCATCGGCTCGACGCCCAAAAAGCACCGAGGTTGAGCCACCCGCC

                                1801                                               1850
AF078102 Rhesus       (1735)    GGAAAGGGTTAGCA------TTTATGACCCCTTGAATGCTAATATTCACT
   M67443 Towne       (1670)    GCGCACGCTTAGGACGACTCTATAAAAACCCACCTCCACTCAGACACGCG

                                1851                                               1900
AF078102 Rhesus       (1779)    GCTTTGATTAAACAATGCATTTGTATTCAATTATCTAATTCCCGT--GTA
   M67443 Towne       (1720)    ACTTTTGGCCGCCA---CACCTGTCGCCGCTGCTATATTTGCCACAGTTG

                                1901                                               1950
AF078102 Rhesus       (1827)    CCGTGTCATTTCTCCATGCAGTGGTGCGAAACACATTACATATTTAGACA
   M67443 Towne       (1767)    CCGGAACCCTTCCCGA--CCTCCCACGAAGACCCGTT-CACCTTTGCGCA

                                1951                                               2000
AF078102 Rhesus       (1877)    -CACGTATCACTTGTGAAAGCTGAGTCAACATCATGCAATTGTCGGACGA
   M67443 Towne       (1814)    TCCCCTGACCCCCCCC----CTCATCCCGCCTTCGCGATGTCTCAGGCAT

                                2001                                               2050
AF078102 Rhesus       (1926)    GGTCCGGGTCCTTCGTTCTGTGTTTCT-GTTACGAGTTTACATTTTATTA
   M67443 Towne       (1860)    CGTCC---TCCCCCGGTGACGCACCCTCGTCGGAAGCGGCCGCGATCAGC

                                2051                      2077
AF078102 Rhesus       (1975)    TGCTTCAAAGTCTTCTATTTCTAA---
   M67443 Towne       (1907)    GAGGCCGAAGCCGCC-AGCGGAAGCTT
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Figure 20: An alignment of the IFN-gamma nucleotide sequences from human, cat, rodent species.

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                                             1                                                50
AF081502 Marmota monax IFN-gamma     (1)    --------------------GGCCTAACTCTCTCTGAAACGATGAAATAG
    D30619 Felis catus IFN-gamma     (1)    CTACTGATTTCAACTTCTTTGGCCTAACTCTC--CGAAACGATGAATTAG
     X87308 Homo sapien IFN-gamma    (1)    --------------------------------------------------

                                             51                                              100
AF081502 Marmota monax IFN-gamma    (31)    ACAAGTTATTTCTTGGCTTTTCAGCTCTGCATCATTTTGGCTTCTTCTAG
    D30619 Felis catus IFN-gamma    (49)    ACAAGTTTTATTTTCGCTTTCCAGCTTTGCATAATTTTGTGTTCTTCTGG
     X87308 Homo sapien IFN-gamma    (1)    --------------------------------------------------

                                             101                                             150
AF081502 Marmota monax IFN-gamma    (81)    CTGTTACTCCCAGGACACAGTTAATAAAGAAATAGAAGATTTAAAAGGAT
    D30619 Felis catus IFN-gamma    (99)    TTATTACTGTCAGGCCATGTTTTTTAAAGAAATAGAAGAGCTAAAGGGAT
     X87308 Homo sapien IFN-gamma    (1)    -TGTTACTGCCAGGACCCATATGTAAAAGAAGCAGAAAACCTTAAGAAAT

                                             151                                             200
AF081502 Marmota monax IFN-gamma   (131)    ATTTTAATGCAAGTAATTCAAATGTATCAGATGGCCGGTCTCTCTTCTTG
    D30619 Felis catus IFN-gamma   (149)    ATTTTAATGCAAGTAATCCAGATGTAGCAGATGGTGGGTCGCTTTTCGTA
     X87308 Homo sapien IFN-gamma   (50)    ATTTTAATGCAGGTCATTCAGATGTAGCGGATAATGGAACTCTTTTCTTA

                                             201                                             250
AF081502 Marmota monax IFN-gamma   (181)    GATATTTTGGATAAATGGAAAGAGGAGAGTGACAAAAAAGTAATCCAGAG
    D30619 Felis catus IFN-gamma   (199)    GACATTTTGAAGAACTGGAAAGAGGAGAGTGATAAAACAATAATTCAAAG
     X87308 Homo sapien IFN-gamma  (100)    GGCATTTTGAAGAATTGGAAAGAGGAGAGTGACAGAAAAATAATGCAGAG

                                             251                                             300
AF081502 Marmota monax IFN-gamma   (231)    CCAAATTGTCTCTTTCTACTTCAAACTCTTTGAACACTTAAAAGACAA--
    D30619 Felis catus IFN-gamma   (249)    CCAAATTGTCTCCTTCTACCTGAAAATGTTTGAAAACCTGAAAGATGATG
     X87308 Homo sapien IFN-gamma  (150)    CCAAATTGTCTCCTTTTACTTCAAACTTTTTAGAAACTTTAAAGATGA--

                                             301                                             350
AF081502 Marmota monax IFN-gamma   (279)    -CAAGATCATCCAAAGGAGCATGGACACCATCAAGGGGGATCTTTTTGCT
    D30619 Felis catus IFN-gamma   (299)    ACCAGCGCATTCAAAGCAGCATGGACACCATCAAGGAAGACATGCTTGAT
     X87308 Homo sapien IFN-gamma  (198)    -CCAGAGCATCCAAAAGAGTGTGGAGACCATCAAGGAAGACATGAATGTC

                                             351                                             400
AF081502 Marmota monax IFN-gamma   (328)    AAGTTCTTCAACAGCAGTACCAATAAGCTGCAGGACTTCCTAAAGGTGTC
    D30619 Felis catus IFN-gamma   (349)    AAGTTGTTAAATACCAGCTCCAGTAAACGGGATGACTTCCTCAAGCTGAT
     X87308 Homo sapien IFN-gamma  (247)    AAGTTTTTCAATAGCAACAAAAAGAAACCAGATGACTTCGAAAAGCTGAC

                                             401                                             450
AF081502 Marmota monax IFN-gamma   (378)    TCAAGTTCAGGTAAATCACCTGAAGATCCAGCCTAAAGCAGTGAGTGAAC
    D30619 Felis catus IFN-gamma   (399)    TCAAATCCCTGTGAATGATCTGCAGGTCCAGCGCAAAGCAATAAATGAAC
     X87308 Homo sapien IFN-gamma  (297)    TAATTATTCGGTAACTGACTTGAATGTCCAACGCAAAGCAATACATGAAC

                                             451                                             500
AF081502 Marmota monax IFN-gamma   (428)    TCAAGAAAGTGATGAATGATCTGTTACCACACTCTACCCTAAGGAAGCCA
    D30619 Felis catus IFN-gamma   (449)    TCTTCAAAGTGATGAATGATCTCTCACCAAGATCTAACCTGAGGAAGCCG
     X87308 Homo sapien IFN-gamma  (347)    TCATCCAAGTGATGGCTGAACTGTCGCCAGCAGCTAAAACAGCGAAGCCG

                                             501                                             550
AF081502 Marmota monax IFN-gamma   (478)    AAAAGGAGTCAGTCTTCGATTCGGGGTCGGAGAGCATCCAAATAACAGTC
    D30619 Felis catus IFN-gamma   (499)    AAAAGGAGCCAGAATCTGTTTCGAGGCCGTAGAGCATCGAAATAATGGTT
     X87308 Homo sapien IFN-gamma  (397)    AAAAGGAGTCAGATGCTGTTTCGAGGTCGAAGAGCATCCCAG--------

                                             551               569
AF081502 Marmota monax IFN-gamma   (528)    CTCATGCCTGC--------
    D30619 Felis catus IFN-gamma   (549)    GTCCTGCCTGCAATATTTG
     X87308 Homo sapien IFN-gamma  (439)    -------------------
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